

**LIGHT AND TRANSMISSION ELECTRON MICROSCOPICAL CHANGES ASSOCIATED WITH GASTRITIS IN TIGER (*PANTHERA TIGRIS*)**

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**ABSTRACT**

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Three dead tigers were dissected and representative samples from the gastric mucosa, duodenum and livers were taken for histopathology and transmission electron microscope for further investigation of the cause of death. Post-mortum examination showed accumulation of straw in the stomach outlet (Phytobezoar), together with pronounced ulcers, which was surrounded by hemorrhagic zones. Light microscopy showed loss of the gastric mucosal epithelium, necrosis of the gastric glands, hyperemia of the submucosal blood vessels, and lymphocytic and few neutrophils in the submucosa. These changes indicated acute-subacute gastritis. Subcapsular hepatocellular necrosis expressed by hepatocellular lysis, pyknosis and karyorhexis of the hepatocytes nuclei were also observed. Transmission electron microscopical observations showed the presence of intercellular, and intercellular elongated, coiled, and spiral form of *Helicobacter* bacteria in close contact with the gastric glandular epithelium. Moreover, rounded-shaped bacteria were observed in association with the *Helicobacter* bacteria. The duodenum revealed also the presence of both types of bacteria. These results suggest mixed infection with the complication of Phytobezoar which cause obliteration of the stomach outlet with chronic constipation and fever, might be responsible for the death of tiger.

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**Key Words:** *Tiger, Helicobacter bacteria, gastritis, Phytobezoar*

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**INTRODUCTION**

The tiger (*Panthera tigris*) is the world's largest cat and is one of the most threatened with extinction. As recently as 100 years ago, there were as many as 100,000 wild tigers living in Asia. Today, fewer than 3,200 remain. Tigers are currently listed as "Endangered" on the International Union of the Conservation of Nature's (IUCN) Red List of Threatened Species (IUCN 2012). Death of tigers in captivity usually related to either fighting or senility but the infectious and non-infectious diseases now become under complete control (personal communication). Phytobezoar is a concretion formed in the stomach or intestine and composed chiefly of undigested compacted vegetable fiber, usually it cure spontaneously by vomiting but in rare case it complicated even to Emphysematous gastritis with perforation of the stomach (Lagios and Suydam 1968). Straw Phytobezoar create a fatal disease in the tigers and lions as obliterate the stomach so complicated to Emphysematous gastritis, gastric ulcers, constipation, fever and death (personal communication). Nausea, vomiting, gastric outlet obstruction, perforation, abdominal pain, and bleeding have been reported in the stomachs of slaughtered plant-eating animals (Chisholm *et al.*, 1992).

**MATERIALS and METHODS**

Three Bengal tiger (*panther tigris*) showed dead between May 2009 and March 2012. Two cases in the Egyptian circus and the last one in the Alexandria private zoo, all revealed symptoms of Nausea, vomiting, fever > 38.8°C (normal temperature 37.5°C or 99.5°F), off food, lethargy, not response to the treatment by antibiotics, antiemetic and stimulant drugs. These animals underwent a thorough post-mortem examinations, Tissues with lesions were removed and fixed in 10% buffered formalin, serial concentrations of alcohol followed by xylene then paraffin-wax embedded for histological examination. 3 µm-thick sections were mounted on slides and stained with haematoxylin-eosin for a general histological view. Direct smear from the gastric and intestinal mucosa take and studied for parasites. In addition advantage examination performed on the last tiger as tissue sections were perfuse using the modified Karnovsky solution, containing 2.5% glutaraldehyde, 2% paraformaldehyde in a 0.1 M (pH 7.3) containing 2.5% sodium cacodylate buffer. Then, the tissues were fixed in the same solution for 12 hours at 4 °C. The phantom of the operate specimens were post fixed in 2% osmium tetroxide solution, rinsed in distilled water, and immersed in 2% tannic acid solution for 1 hour at room temperature (Murakami, 1974). Then, the tissues were dehydrated

in an increasing series of ethanol and propylene oxide and embedded in Spurr resin. The thick sections were made in Porter Blum ultra microtome using glass knives and stained with toluidin blue solution for light microscopy analysis. For thin sections were used ultra microtome Ultra-Cut Reichert with diamond knife. The ultrathin sections were mounted on 200 and 300 mesh grids, counterstained with uranyl acetate and lead citrate and examined in transmission electron microscopy Joel, JSM1010at 100 kV.

## RESULTS

Accumulation of straw in the stomach of dead tiger was observed in the form of ball-like structure (Phytobezoar) during postmortem examination (Fig.1c). Moreover, pronounced circumscribed ulcers were observed and surrounded by hemorrhagic zone (Fig.1d).

### I. Light microscopy:

#### A. Stomach:

Histopathological examination of the tiger stomach showed ulcers of its mucosa, which was expressed by loss of the surface epithelium of the mucosa as well as degenerative and necrotic changes of the gastric glands (Figs. 2;3). Fig.4 showed hyperemia of the submucosal blood vessels, which was expressed by dilation and engorgement with blood. Within the hyperemic blood vessels several vacuoles were observed in close contact with the endothelium of

tunica intima (Fig.5). Pronounced inflammatory cellular reaction mainly of lymphocytes and neutrophils were also observed in the submucosa (Fig.6).

#### B. Liver:

Sub-capsular hepatocellular necrosis was observed (Fig.7).This necrotic area was expressed by hepatocellular lysis, pyknosis, and karyorehesis of nuclei (Figs. 8;9).

## II. Transmission Electron microscope:

#### A. Stomach:

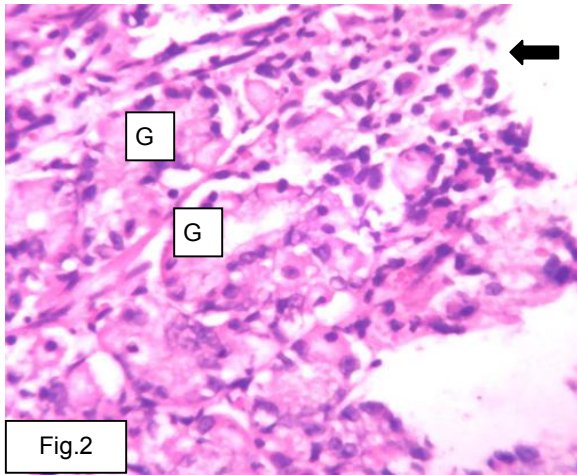
Abundant electron dense structures in close contact with the gastric glandular epithelium was noticed (Fig.10).These structures were elongated, helical-like or spiral shape belong to *Helicobacter* spp.(Fig.11). These bacteria were also observed between the gastric glands near the gastric pet (Fig.12). In addition to the spiral helicobacter bacteria, rounded-shaped electron dense bacteria were also observed between the gastric glands (Fig.13). Intracellular localization of the bacteria was observed in close contact to the mitochondria (Fig.14).

#### B. duodenum:

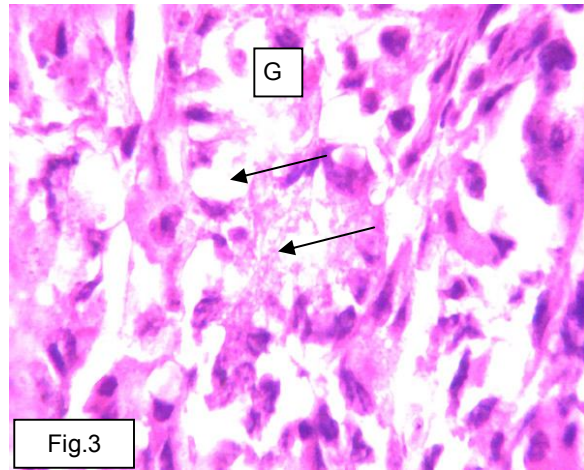
Figs. 15;16 showed intracellular localization of the electron-dense rounded bacteria surrounded by a halo and inside phagolysosomes. A coiled spiral helicobacter bacteria and the rounded electron-dense bacteria were also observed intracellular (Fig.17).



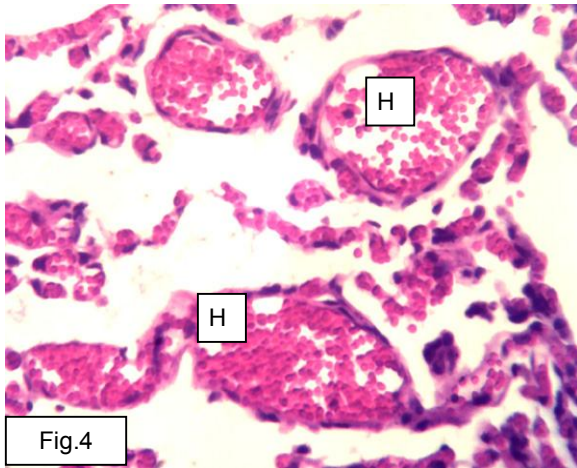
**Fig.1:** A tigress with her cubs on straw (a). Adult Dead Tiger during PM showing the complete dentation (b). Stomach had accumulation of straw (Thick arrow) and few ulcers (small arrows) (c). Pronounced circumscribed ulcers surrounded by hemorrhagic zone (Bottom arrow) with duodenal necrosis (red arrow) (d).



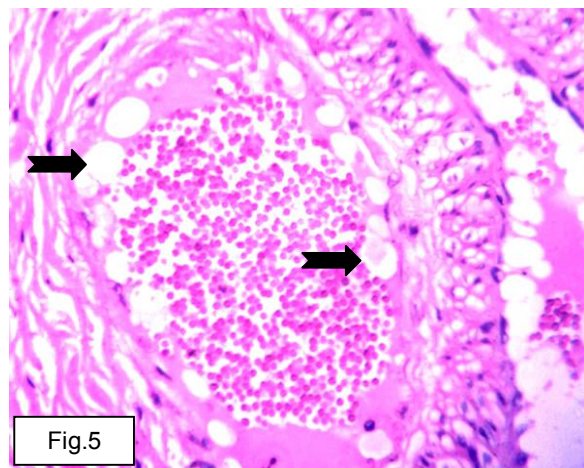
**Fig.2:** Tiger stomach showing loss of surface epithelium of the mucosa (arrow) and degeneration of gastric glands (G).X40.



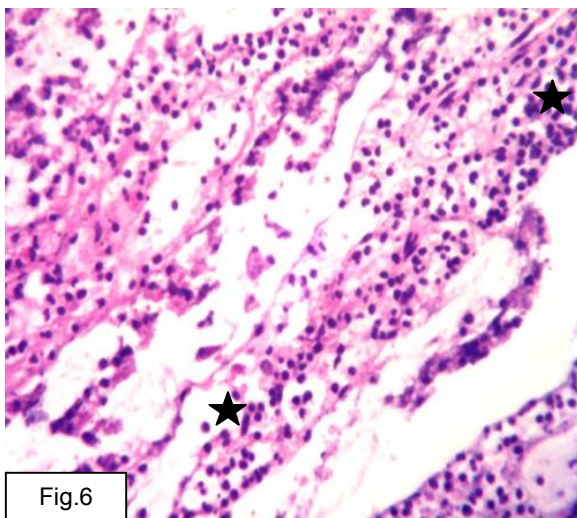
**Fig.3:** Higher magnification of Fig.2 showing necrosis of gastric glands (arrow). H&E. X40.



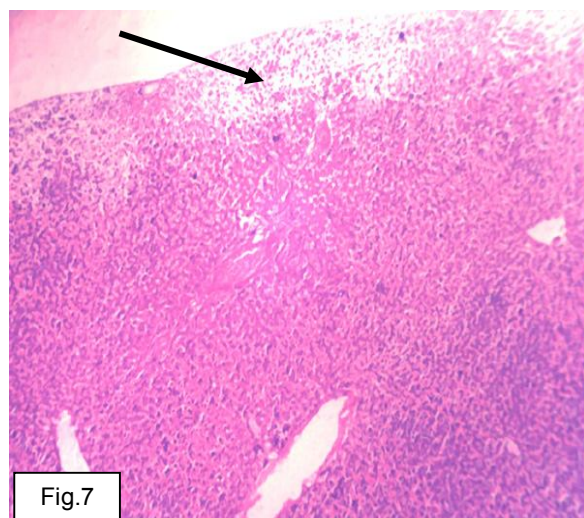
**Fig.4:** Tiger stomach showing hyperemia of submucosal H&E. X40.



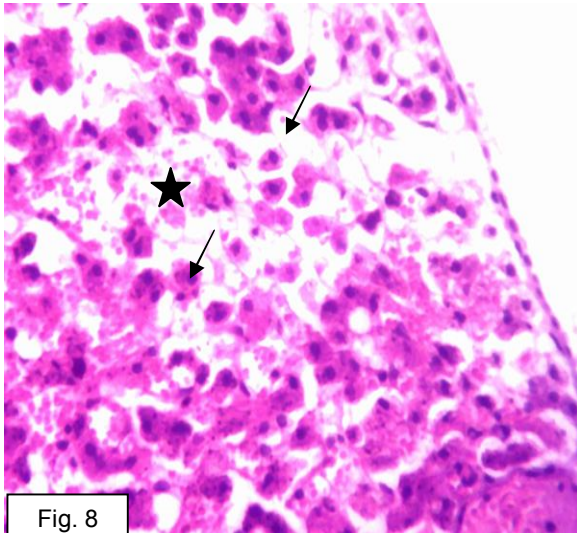
**Fig.5:** Tiger stomach showing hyperemic blood vessel with accumulation of several vacuoles (head arrow). X40



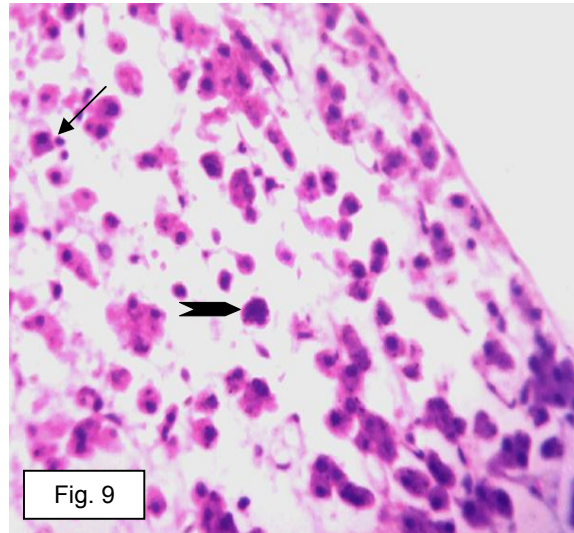
**Fig.6:** Tiger stomach showing inflammatory cellular reaction. (\*). H&E.X10



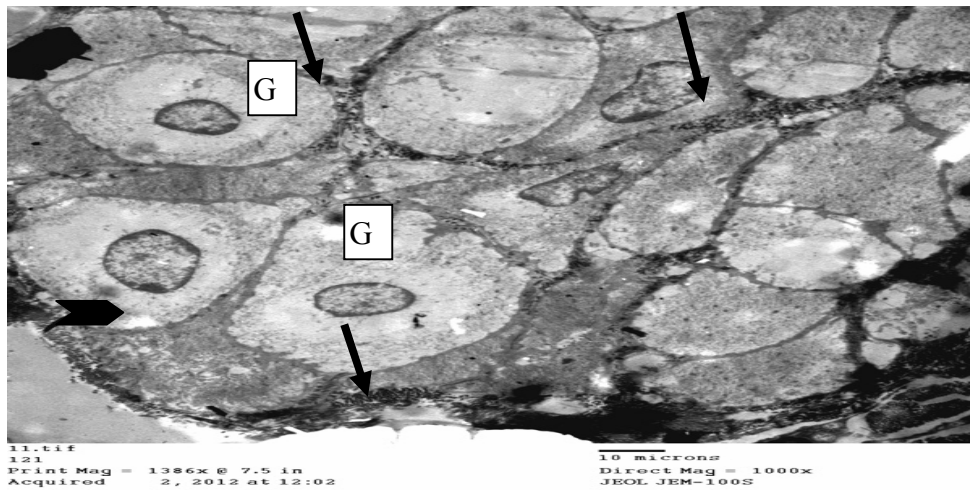
**Fig7:** Tiger liver showing subcapsular necrosis (arrow).X10



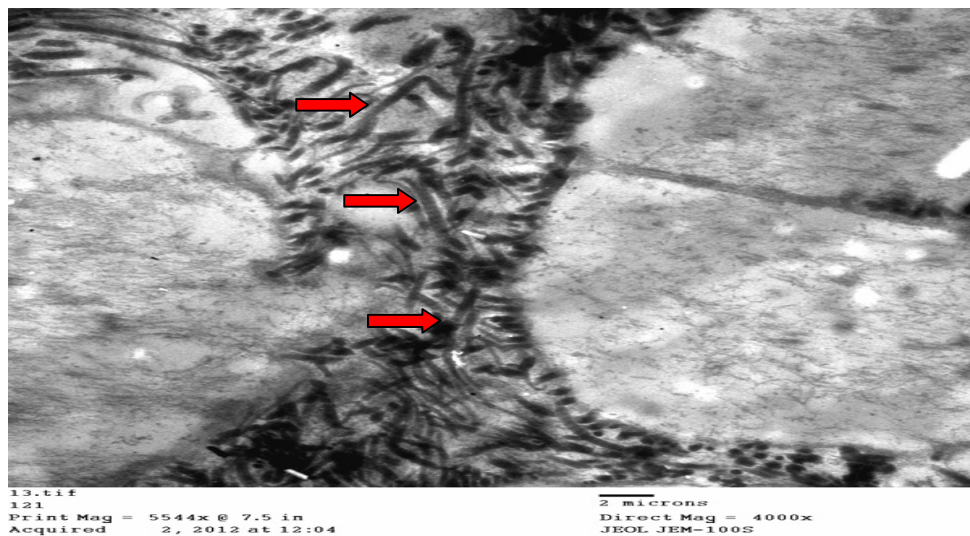
**Fig. 8:** Higher Magnification of Fig.7 showed hepatocellular Lysis (star), and pyknotic nuclei (arrow). X40.



**Fig. 9:** Tiger liver showed hepatocellular lysis and nuclear Changes (pyknosis, Arrow; Karyorhexis, Head arrow). H.&E.X40.



**Fig.10:** Electron-dense structures (arrow) in close contact with the gastric glandular epithelium (G). Bar=10um.



**Fig.11:** Higher magnification of Fig.10 showed elongated spiral-shaped bacteria (Helicobacteria sp.) (Arrow). Bar=2um

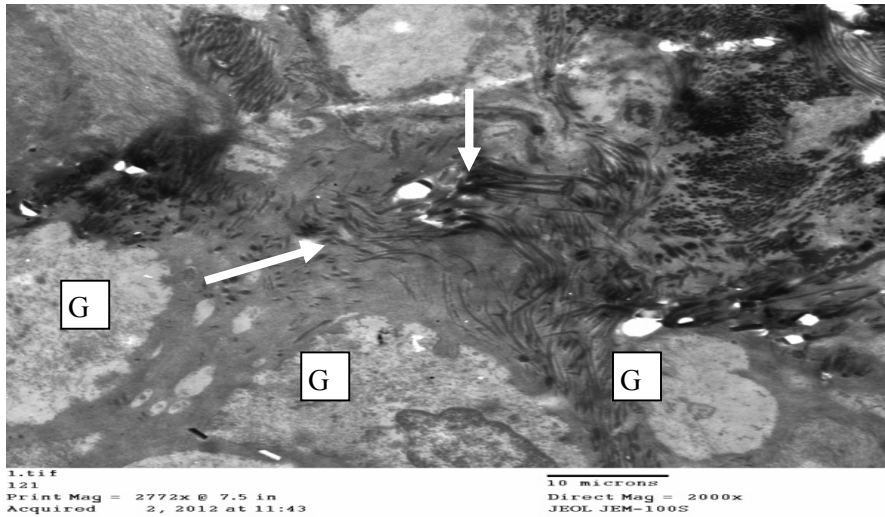


Fig. 12: TEM of tiger stomach showing spiral-shaped bacteria (arrow) between the gastric glandular Epithelium (G).bar=10um.

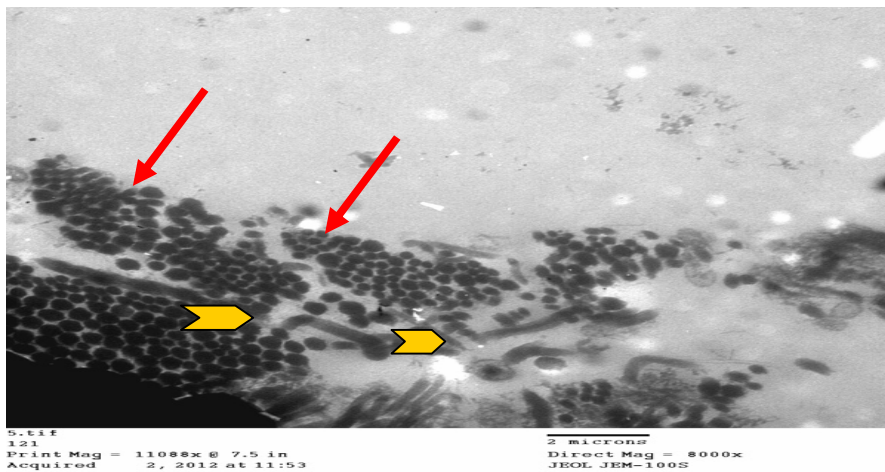


Fig.13: Higher Magnification of Fig. 12 showing spiral-shape bacteria (Head arrow) as well as rounded-shape (Arrow). Bar=2um.

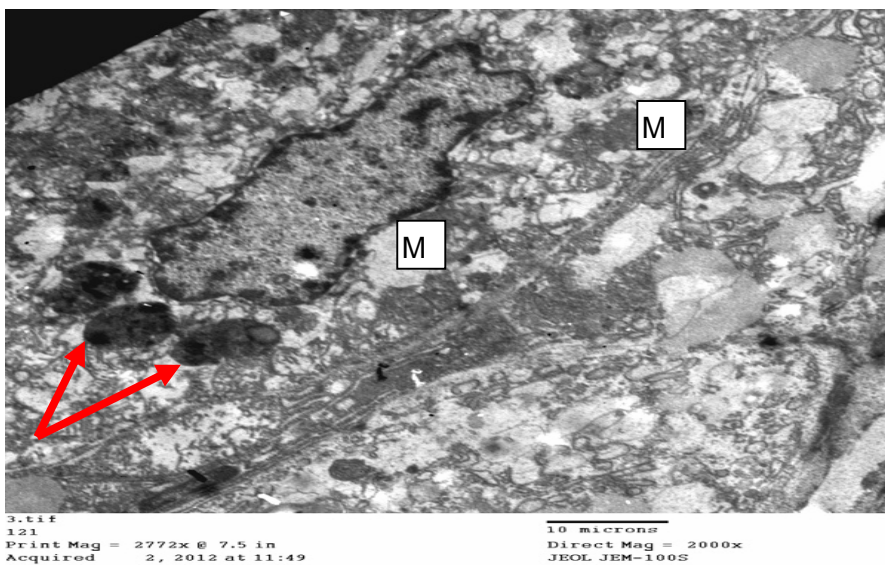
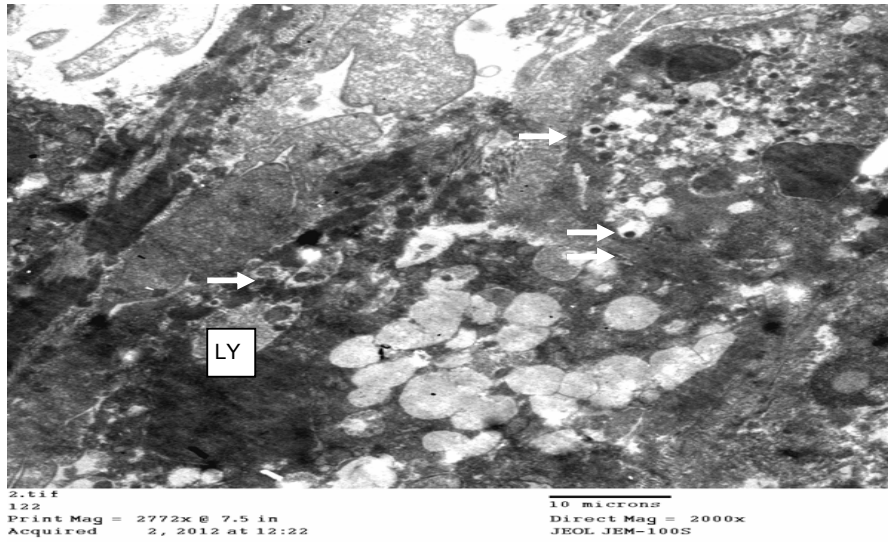
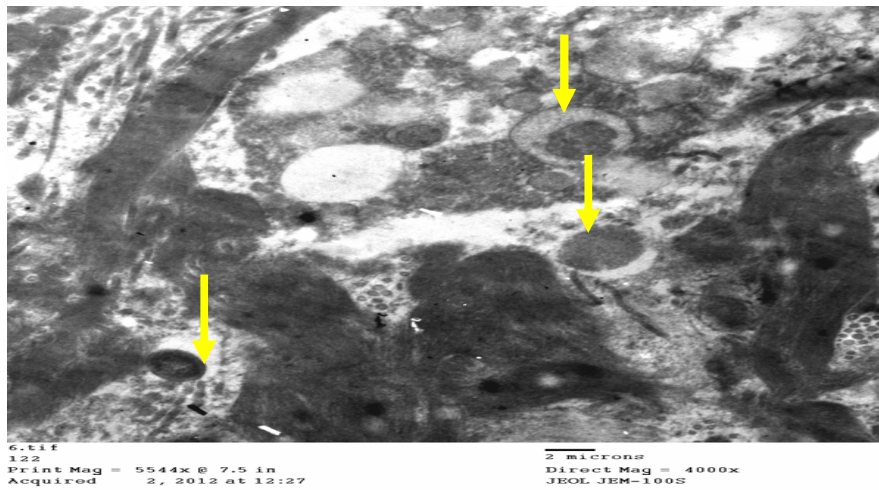


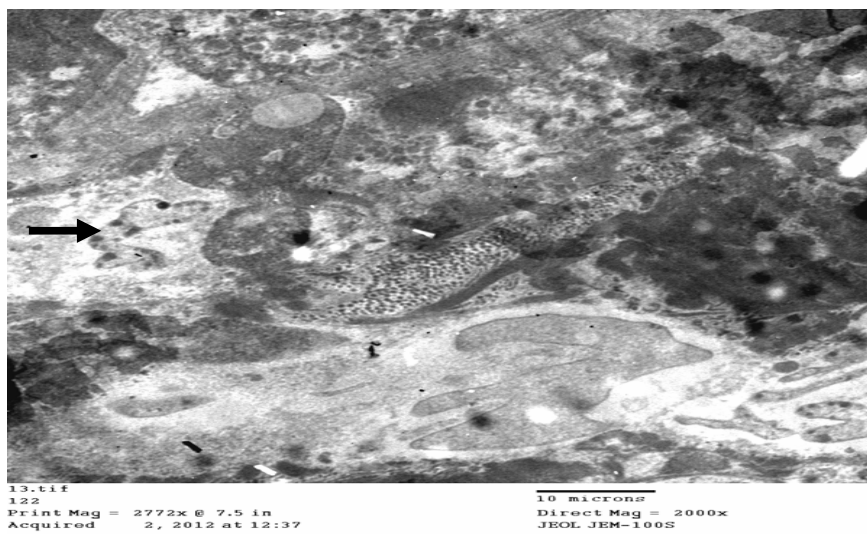
Fig.14: TEM of tiger stomach showing intracellular localization of bacteria (arrow) close to the mitochondria (M). Bar=10um.



**Fig.15:** TEM of tiger doudenum showing intracellular localization of bacteria (arrow) surrounded by a hallow inside phagolysosomes (Ly). Bar= 10um.



**Fig.16:** Higher magnification of Fig.15. Bar= 2 um.



**Fig.17:** TEM of tiger doudenum showing intracellular localization of coiled spiral form of bacteria. Bar= 10um. (arrow)

## **DISCUSSION**

In the present study post-mortum examination of the dead tiger revealed accumulation of straw in the inlet of the stomach. This condition in zoo animals is known as Phytobezoar.

(Barrs *et al.*, 1999) recorded trichobezoars in five domestic crossbred cats (four long haired, one short haired. Phytobezoar recorded before in horses by (Banse *et al.*, 2011 and MacDonald 1987). A juvenile male vervet monkey was found with a phytobezoar of straw obstructing the sigmoid colon also recorded in rhesus monkey (*Macaca mulatta*) (Kessler, and Kupper 1976).

Pronounced ulcers in the stomach was observed and surrounded with hemorrhagic zone.

Histopathological examination of tiger stomach in the present study showed different components of inflammation. Loss of mucosal surface epithelium and reached up to the superficial mucosal gastric glands was observed and explained the ulcer formation observed grossly. Moreover, necrosis of the gastric glands was evident. These changes represented the alterative component of inflammation. Hyperemia of the submucosal blood vessels was observed and suggested acute inflammation of the stomach occurred. Moreover, the vacuolated appearance of tunica intima endothelium may suggest alter membrane permeability. These changes together with the inflammatory cellular reaction suggest acute-subacute gastritis.

The present study showed subcapsular necrosis of the liver and manifested by hepaocellular lysis, as well as nuclear changes expressed by pyknosis and karyorehexis. Harbour and Sutton (2008) reported that *Helicobacter hepaticus* was originally isolated from the livers of mice suffering from multifocal necrotic hepatitis. It has since been found to colonize both the intestine and, less frequently, the liver of mice and is prevalent in many mouse colonies.

Transmission Electron Microscopy (TEM) in the present study showed the presence of elongated spiral coiled bacteria and suggested *Helicobacter* sp. Several studies have shown the features of these bacteria (Harbour and Sutton, 2008; Yamamoto *et al.*, 2011). Moreover, the present study revealed that these bacteria were in close contact with the gastric glandular epithelium, intercellular and intracellular. These results suggest that *Helicobacter* sp. is the main primary etiology responsible for the death of tiger due to acute gastritis. However, several rounded bacteria were also observed in association with the

*Helicobacter*. These changes suggest that mixed infection occurred.

Schoroder *et al.* (1997) stated that *Helicobacter acinonyx* from tigers, in which, as previously reported in cheetahs, it may be a cause of gastritis. Harbour and Sutton (2008) stated that in addition to infection of small domestic animals, there are numerous reports of *Helicobacter*-like organisms in a wide range of large cats and dogs, including lion, tiger, hyena, wolf, fox, bobcat, while these were not properly characterized. Yamamoto *et al.* (2011) stated that *Helicobacter pylori* (*H. pylori*), which is a gram-negative bacterium with a slightly curve-shaped morphology, colonizes the human stomach. Some non-*H. pylori* *Helicobacter* (NHPH) found in the human stomach are sometimes observed in other animal species, such as cats, dogs, pigs, and primates. Therefore, human infections are suspected to be caused through human contact with these animals, and the possibility of the zoonotic transmission of animal *Helicobacter* to humans is a common concern. *H. pylori* is considered to be a major factor in the development of chronic atrophic gastritis, peptic ulcers, and gastric cancer (Yamamoto *et al.*, 2011). Transmission electron microscopy revealed slightly curved organisms, 2-3um in length and 0. 0-5 um in diameter, in the gastric glands of the superficial mucosa. The bacteria were often found in close contact with the cell membrane of the gastric epithelial cells. In morphology and location, these organisms were similar to *H. pylori* of man and to *H. acinonyx* of cheetahs (Schoroder *et al.*, 1997).

Although, reports indicated the absence of *Helicobacter* bacteria in the duodenum (Schoroder *et al.*, 1997; Harbour and Sutton, 2008). TEM in the present study showed the presence of coiled form and elongated bacteria as well as bacteria surrounded by hallow. Moreover, these bacteria were seen inside phagolysosomes.

In conclusion, the present study showed the dangerous of using the straw as bedding in the food rooms, *Helicobacter* bacteria observed as the primary etiology for gastritis in association with other rounded bacteria suggesting mixed infection occurred as a complication of the Phytobezoar and responsible for the death of tiger. Therefore, zoo keepers were recommended to avoid the use of straw as litter in the food rooms.

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## استخدام الميكروسكوب الضوئي والالكتروني القاطع لدراسة التغيرات المصاحبة لالتهاب المعدة لدى النمر البنغالي

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اجري التشريح المرضي على ثلاث نمور بنغالي وأخذت عينات ممثلة من المعدة والأمعاء والكبد والرتنين للفحص الباثولوجي باستخدام الميكروسكوب الضوئي وبالإضافة للميكروسكوب الالكتروني القاطع على الحالة الثالثة لبيان أسباب النفوق. أوضح التشريح المرضي وجود تجمع من قش الأرز على عند مدخل الاثنى عشر مع وجود تقرحات عديدة بجدار المعدة وبقع نزيفية. اظهر الفحص المجهرى فقدان الجدار المبطن للمعدة وتتركز النسيج المخاطي مع احتقان النسيج تحت المخاطي وتواجد بعض خلايا النيوتروفيل. هذه التغيرات تدل على التهاب معدي حاد وتحت حاد. هذا بالإضافة إلى وجود بقع تنكزية تحت كبسولة الكبد. بالاستعانة بالميكروسكوب الاليكتروني القاطع تلاحظ تواجد أعداد كبيرة من باكتيريا الهليكوباكتر الحلزونية تحيط بخلايا الغشاء المبطن للمعدة والاثنى عشر بالإضافة إلى تواجده أيضا داخل الخلايا المناعية ، هذا بالإضافة إلى تواجد أعداد كبيرة من البكتيريا الكروية. يتضح من الدراسة أن أسباب النفوق هو الإصابة الميكروبية نتيجة لمضاعفات انسداد المعدة الذي أدى إلى إمساك مزمن ثم حمى أودت بحياة الحيوان.